Supplemental Information

Figure legends

Figure 1

(A) Cell growth activity measured by MTT assay showed similar results to the results obtained by luciferase assay. After the 3-days incubation of CM derived from PNT-2 cells or PC-3M-luc cells with PC-3M cells, inhibitory effect of PC-3M-luc cellular proliferation was assessed by MTT assay and luciferase assay. Each bar is presented as mean S.E. (n=3). (**, $p<0.005$; Student's *t* test) (B) Purified exosomes secreted from PNT-2 cells are enriched in CD63 protein. The exosome fractions collected from PNT-2 derived CM incubated for 0 hr or 48 hr and whole cell lysate of PNT-2 cells were analyzed by immunoblotting with an anti-CD63 antibody or anti-β-actin antibody. Equal amounts of proteins from exosome and whole cells were analyzed. Twenty microlitter of exosome samples derived from 0 h incubation and 48 h were also analyzed. CD63 is heavily glycosylated displaying a range of bands between 50 and 75 kDa. (C) After the treatment with the 10 μM of GW4869 for 48 hr, the total amounts of proteins in the exosomal pellet purified from large scale cultures of PNT-2 cells were quantified by a BCA assay and are presented as the values per 10 million secreting cells. Each bar is presented as mean S.E. (n=3). (**, $p<0.005$; Student's *t* test) (D) Fluorescence photos of PC-3M-luc cells incorporating PKH67-loaded exosomes isolated from PNT-2 cells. PC-3M-luc cells were incubated for 0 hr and 16 hr with PKH67-loaded exosomes. Emission at 514 nm was detected with Eclipse TE 2000 Inverted Research Microscope and images were produced by NIS-Elements BR software. The size bar indicates 50 μm.

Figure 2

(A) GW4869 treatment of PNT-2 did not affect the expression of miR-143 in the cells. PNT-2 cells were treated with 10 μM of GW4869 in a 6-well plate. One day later, medium were changed to non-serum medium. The following day, cell extract were applied to QRT-PCR for miR-143. The values on the *y* axis are depicted relative to the amount of miRNAs of control, which is arbitrarily defined as 1. (B) siRNA-mediated knockdown of nSMase2 in PNT-2 cells. PNT-2 cells were transfected with either negative control or nSMase 2 siRNA in a 24-well plate. One day later, cell extract were applied to QRT-PCR for nSMase 2. The values on the *y* axis are depicted relative to the amount of nSMase 2 of control, which is arbitrarily defined as 1. (**, $p<0.005$; Student's *t* test) (C) and (D) siRNA-mediated knockdown of nSMase 2. PNT-2 cells

were transfected with either negative control or nSMase2 siRNA in a 24-well plate. The following day, conditioned medium (C) and cell extract (D) were applied to QRT-PCR for miR-143. The values on the *y* axis are depicted relative to the amount of miRNAs of control, which is arbitrarily defined as 1. (**, $p<0.005$; Student's *t* test)

Figure 3

(A) miR-143 were secreted into culture medium. Conditioned medium from miR-143 stably expressing HEK293 cells or control non-transfected HEK293 cells were harvested and performed QRT-PCR as described under Materials and methods. The values on Y-axis are depicted relative to the amount of miR-143 in conditioned medium from non-transfected HEK293 cells, which is arbitrarily defined as 1. (B) PC-3M-luc cells were incubated with CM derived from HEK293 cells or over-producing miR-143 HEK293 cells for 24 hr at 37ºC and the transfer of miR-143 was evaluated by qRTPCR. The values on the *y* axis are depicted relative to the normalized miR-143 expression of original HEK293 CM-treated cells, which is defined as 1. Each *bar* is presented as the mean S.E. ($n=3$). (*, $p<0.05$; Student's *t* test)

Fig. 1

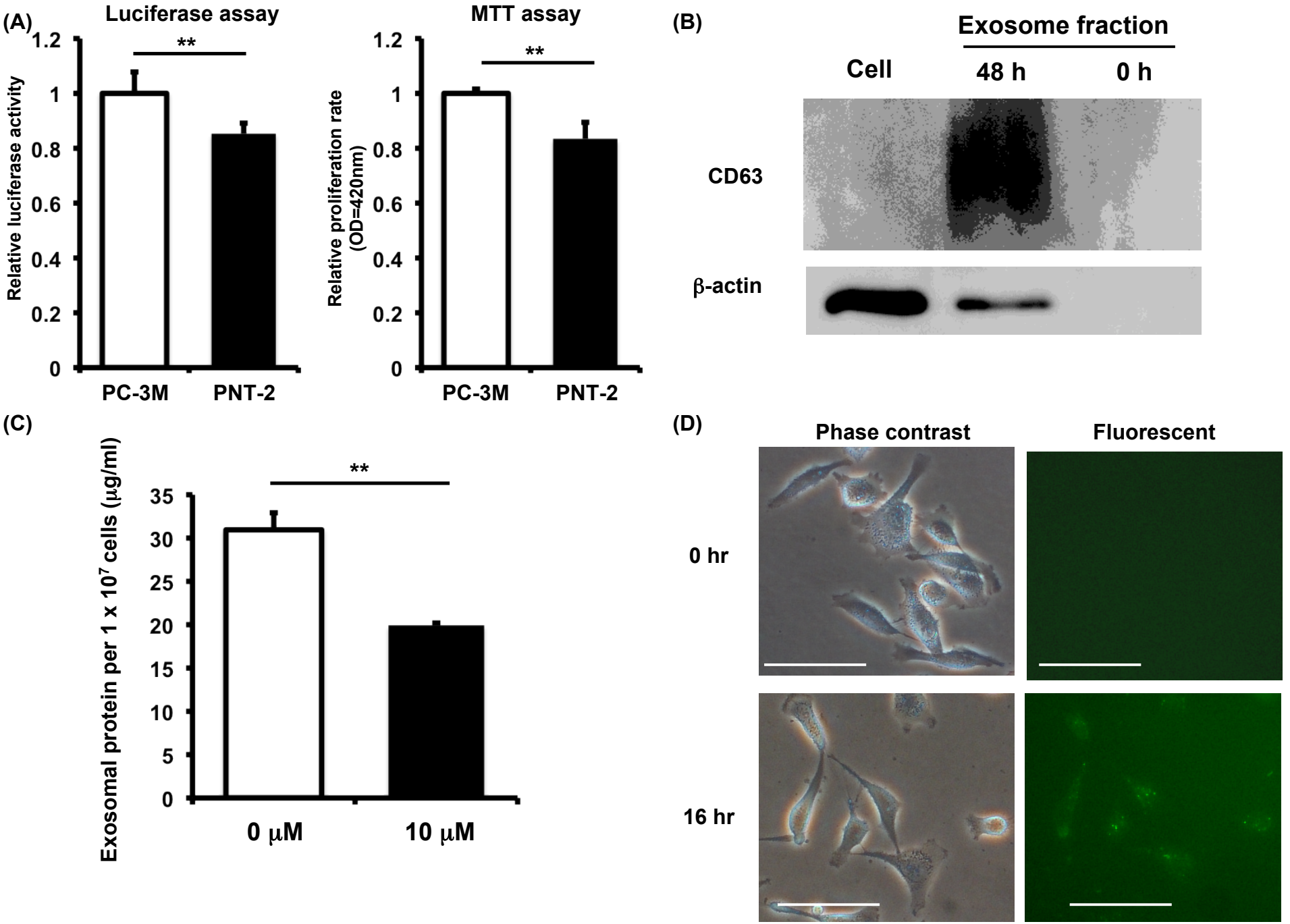

(A)
Luciferase assay
MTT assay
Relative luciferase activity
Relative proliferation rate (OD=420nm)
**
**
1.2
1
0.8
0.6
0.4
0.2
0
PC-3M
PNT-2
(B)
Exosome fraction
Cell
48 h
0 h
CD63
β-actin
(C)
Exosomal protein per 1 x 10^7 cells (μg/ml)
**
35
30
25
20
15
10
5
0
0 μM
10 μM
(D)
Phase contrast
Fluorescent
0 hr
16 hr

Fig. 2

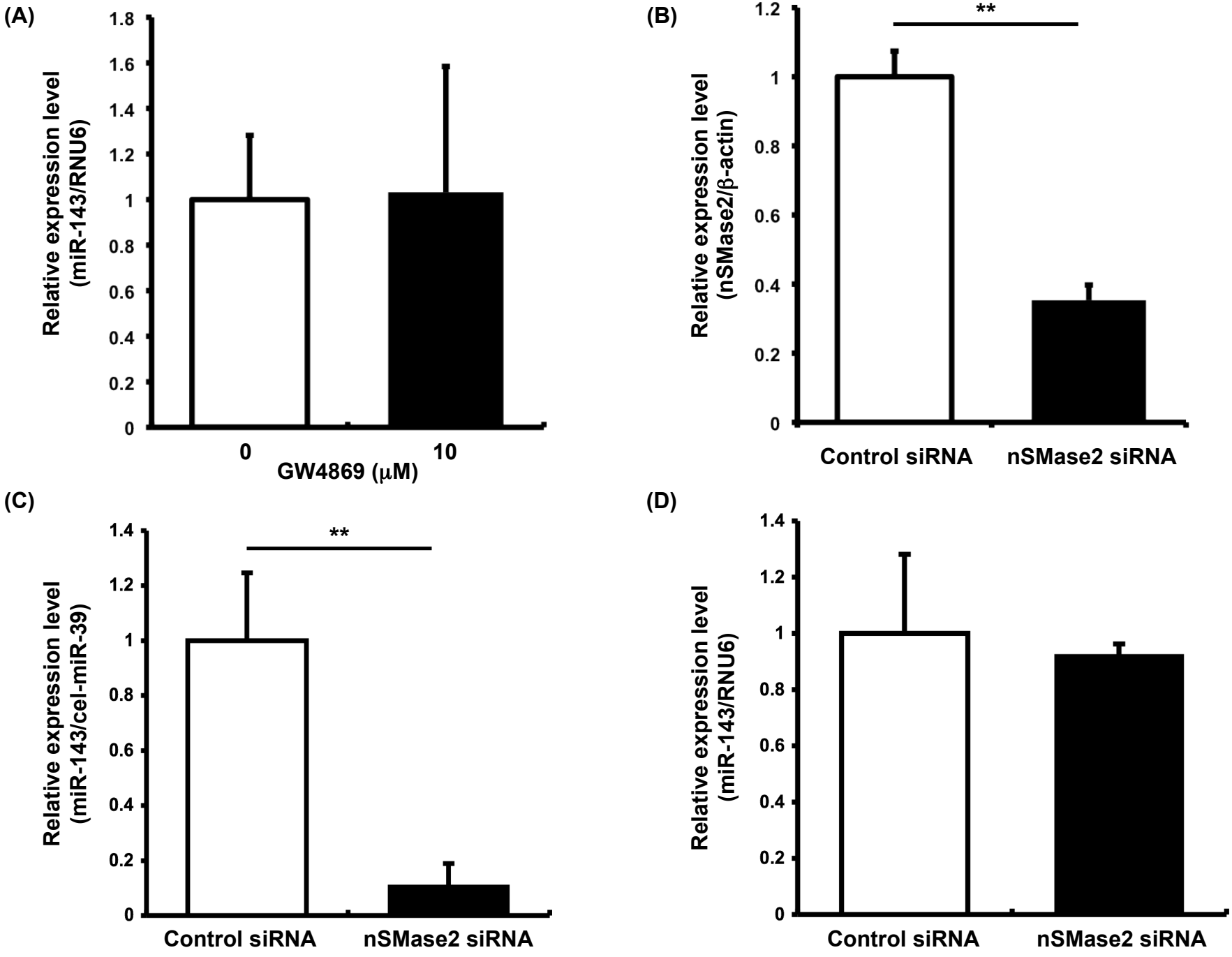
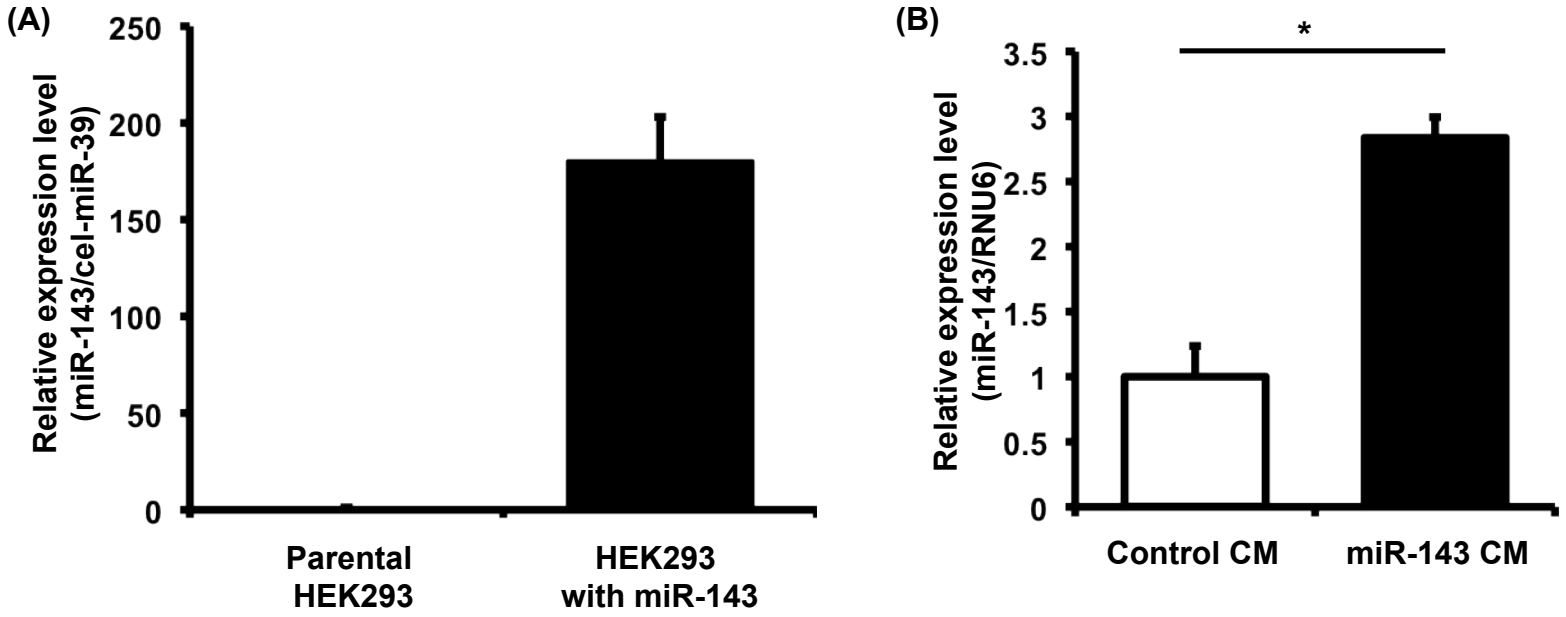

(A)
Relative expression level (miR-143/RNU6)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
10
GW4869 (μM)
(B)
Relative expression level (nSMase2/β-actin)
**
1.2
1
0.8
0.6
0.4
0.2
0
Control siRNA
nSMase2 siRNA
(C)
Relative expression level (miR-143/cel-miR-39)
**
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control siRNA
nSMase2 siRNA
(D)
Relative expression level (miR-143/RNU6)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control siRNA
nSMase2 siRNA

Fig. 3


(A)
Relative expression level
(miR-143/cel-miR-39)
250
200
150
100
50
0
Parental
HEK293
HEK293
with miR-143
(B)
*
Relative expression level
(miR-143/RNU6)
3.5
3
2.5
2
1.5
1
0.5
0
Control CM
miR-143 CM